**PCT** WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : A61L 33/08, 27/34, 31/10 — A1

(11) International Publication Number: WO 00/56377

(43) International Publication Date: 28 September 2000 (28.09.00)

(21) International Application Number: PCT/US00/07228

(22) International Filing Date: 17 March 2000 (17.03.00)

(30) Priority Data:

| | | |
|---|---|---|
| 60/125,355 | 19 March 1999 (19.03.99) | US |
| 09/430,857 | 29 October 1999 (29.10.99) | US |


(71) Applicant *(for all designated States except US)*: GENZYME CORPORATION [US/US]; Metrowest Place (MWP), P.O. Box 9322, Framingham, MA 01701 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: WAN, Barbara [US/US]; 1915 Maple Avenue, Evanston, IL 60201 (US). MILLER, Robert, J. [US/US]; 59 Fairway Drive, Halifax, MA 02338 (US).

(74) Agent: COBERT, Robert, J.; Genzyme Corporation, Legal Department, 15 Pleasant Street, Framingham, MA 01701-9322 (US).

(81) Designated States: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

**Published**

*With international search report.*

*Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.*

(54) Title: SURFACE MODIFICATION OF SUBSTRATES

(57) Abstract

Methods of improving the surface characteristics of a medical device or instrument, and the devices and instruments improved thereby, are provided. The disclosed methods involve the chemical attachment of an activated hyaluronic acid, such as hyaluronic acid reacted with a carbodiimide, onto the surface of the device. The hyaluronic acid can be modified to include the presence of $Fe_{+3}$ or $Al_{+3}$ cations to prevent calcification of the device and surrounding tissue. The devices and instruments are particularly useful during a surgical procedure.

*FOR THE PURPOSES OF INFORMATION ONLY*

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **AL** | Albania | **ES** | Spain | **LS** | Lesotho | **SI** | Slovenia |
| **AM** | Armenia | **FI** | Finland | **LT** | Lithuania | **SK** | Slovakia |
| **AT** | Austria | **FR** | France | **LU** | Luxembourg | **SN** | Senegal |
| **AU** | Australia | **GA** | Gabon | **LV** | Latvia | **SZ** | Swaziland |
| **AZ** | Azerbaijan | **GB** | United Kingdom | **MC** | Monaco | **TD** | Chad |
| **BA** | Bosnia and Herzegovina | **GE** | Georgia | **MD** | Republic of Moldova | **TG** | Togo |
| **BB** | Barbados | **GH** | Ghana | **MG** | Madagascar | **TJ** | Tajikistan |
| **BE** | Belgium | **GN** | Guinea | **MK** | The former Yugoslav Republic of Macedonia | **TM** | Turkmenistan |
| **BF** | Burkina Faso | **GR** | Greece | | | **TR** | Turkey |
| **BG** | Bulgaria | **HU** | Hungary | **ML** | Mali | **TT** | Trinidad and Tobago |
| **BJ** | Benin | **IE** | Ireland | **MN** | Mongolia | **UA** | Ukraine |
| **BR** | Brazil | **IL** | Israel | **MR** | Mauritania | **UG** | Uganda |
| **BY** | Belarus | **IS** | Iceland | **MW** | Malawi | **US** | United States of America |
| **CA** | Canada | **IT** | Italy | **MX** | Mexico | **UZ** | Uzbekistan |
| **CF** | Central African Republic | **JP** | Japan | **NE** | Niger | **VN** | Viet Nam |
| **CG** | Congo | **KE** | Kenya | **NL** | Netherlands | **YU** | Yugoslavia |
| **CH** | Switzerland | **KG** | Kyrgyzstan | **NO** | Norway | **ZW** | Zimbabwe |
| **CI** | Côte d'Ivoire | **KP** | Democratic People's Republic of Korea | **NZ** | New Zealand | | |
| **CM** | Cameroon | | | **PL** | Poland | | |
| **CN** | China | **KR** | Republic of Korea | **PT** | Portugal | | |
| **CU** | Cuba | **KZ** | Kazakstan | **RO** | Romania | | |
| **CZ** | Czech Republic | **LC** | Saint Lucia | **RU** | Russian Federation | | |
| **DE** | Germany | **LI** | Liechtenstein | **SD** | Sudan | | |
| **DK** | Denmark | **LK** | Sri Lanka | **SE** | Sweden | | |
| **EE** | Estonia | **LR** | Liberia | **SG** | Singapore | | |

# SURFACE MODIFICATION OF SUBSTRATES

## BACKGROUND OF THE INVENTION

This invention relates to methods for inhibiting the adherence of platelets and certain cell types to the surfaces of medical devices and instruments which come into contact with platelets and cells, and for preventing mineralization of such devices and instruments. This is accomplished by chemically bonding a modified or derivatized hyaluronic acid molecule to the device or instrument, and by the addition of $Fe^{+3}$ or $Al^{+3}$ cations to the hyaluronic acid.

When a blood vessel is damaged and the normal endothelial-cell barrier is disrupted, platelets are quickly recruited from the circulating blood to form an occlusive plug. This occurs through a series of interactions between the platelets and macromolecules in the subendothelial matrix (platelet adhesion) and among the platelets themselves (platelet aggregation). The initial process of adhesion, in contrast to aggregation, does not require metabolic activity. It leads, however, to the activation of platelets which in turn secrete a number of factors which stimulate the activation of plasma coagulation factors, resulting in the generation of a fibrin clot that reinforces the platelet aggregate.

Under normal hemostatic conditions, the platelet aggregate and fibrin clot are degraded as healing of the injured area occurs. However, a platelet aggregate and/or a fibrin clot can occlude a blood vessel in a pathological process known as thrombosis. Venous thrombosis and pulmonary embolism are among the leading causes of morbidity and death in hospitalized patients.

Thrombosis that develops as a purely intravascular process may also be the primary factor in atherosclerosis. The formation of platelet aggregates on the surface of atheromatous plaques and subsequent organization of these white thrombi into fibrous occlusive intimal lesions may be one mechanism by which atherosclerotic lesions progress to severe obstruction and total occlusion; coronary artery thrombosis leading to myocardial infarction almost always occurs at the site of an atheromatous plaque. Percutaneous transluminal coronary angioplasty (PTCA) has become an important procedure for re-establishing blood flow to the heart through partially occluded blood vessels. Unfortunately, approximately 30% to 40% of patients that have coronary angioplasty suffer restenosis of the treated vessel within 6 months of treatment.

U.S. Patent No. 5,585,361 describes the use of unmodified hyaluronic acid to inhibit platelet adherence and aggregation. The hyaluronic acid can be administered to a patient intravenously, or the hyaluronic acid can be used as a coating on a prosthetic device, such as a vascular graft, a heart valve, a vascular stent or a catheter. This patent does not disclose that the hyaluronic acid can be chemically attached to the surface of the prosthetic device, or, in fact, that the hyaluronic acid can be used in a form other than its native form, such as in a cross-linked or derivatized form.

Hyaluronic acid ("HA") is a naturally occurring mucopolysaccharide found, for example, in synovial fluid, in vitreous humor, in blood vessel walls and the umbilical cord, and in other connective tissues. The polysaccharide consists of alternating N-acetyl-D-glucosamine and D-glucuronic acid residues joined by alternating β 1-3 glucuronidic and β 1-4 glucosaminidic bonds, so that the repeating unit is -(1→4)-β-D-GlcA-(1→3)-β-D-GlcNAc-. In water, hyaluronic acid dissolves to form a highly viscous fluid. The molecular weight of hyaluronic acid isolated from natural sources generally falls within the range of $5 \times 10^4$ up to $1 \times 10^7$ daltons.

Hyaluronic acid, in chemically modified ("derivatized") form, is useful as a surgical aid to prevent adhesions or accretions of body tissues during the post-operation period. The derivatized HA gel or film is injected or inserted into the locus between the tissues that are to be kept separate to inhibit their mutual adhesion. Chemically modified HA can also be useful for controlled release drug delivery. See U.S. Patent No. 4,937,270 and U.S. Patent 5,017,229 which disclosed derivatized versions of HA prepared, for instance, by reacting HA with a carbodiimide.

I. Danishefsky et al., *Carbohydrate Res.*, Vol. 16, pages 199-205, 1971, describe the modification of a mucopolysaccharide by converting the carboxyl groups of the mucopolysaccharide into substituted amides by reacting the mucopolysaccharide with an amino acid ester in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride ("EDC") in aqueous solution. Danishefsky et al. react glycine methyl ester with a variety of polysaccharides, including HA. The resulting products are water soluble; that is, they rapidly disperse in water or in an aqueous environment such as is encountered between body tissues.

U.S. Patent No. 4,582,865 and U.S. Patent No. 4,636,524 describe HA compositions in which the HA is cross-linked by reaction with divinyl sulfone, and the use of these compositions for drug delivery applications. U.S. Patent No. 5,676,964 describes the

preparation of cross-linked polysaccharides, including HA, wherein the cross-linking occurs as a result of covalent bonds formed between carboxyl groups and hydroxyl groups of adjacent polysaccharide molecules. Johns et al., *Fertility and Sterility,* Vol. 68, pages 37-42 (1997), disclose hyaluronic acid formulations crosslinked with trivalent iron for use in reducing adhesion formation.

A heparin-like molecule is described in R. Barbucci et al., *Society for Biomaterials Meetings,* Abstracts (1998), which also describes the immobilization of the molecule on the surface of different materials, including polyethyleneterephthalate ("PET"), to study blood compatibility using various immobilization procedures, such as photolithography immobilization, glow discharge grafting, and PUPA immobilization. The heparin-like molecule contains the backbone of hyaluronic acid, with sulfate groups replacing the hydroxyl groups. The blood compatibility of the immobilized PET substrate was evaluated with respect to anticoagulant activity, platelet adhesion and thrombus formation, and coagulation factor activity. See also P. Favio et al., *Immobilization of heparin and highly-sulphated hyaluronic acid onto plasma-treated polyethylene,* Plasmas and Polymers, Vol. 3, No. 2 (1998), which describes the immobilization of heparin and highly-sulphated hyaluronic acid onto plasma-processed polyethylene, using a diamine polyethylene glycol spacer molecule, to improve the compatibility of the polyethylene with human blood. Both a treatment and a deposition process is used to provide -COOH groups on the polyethylene surface.

The use of polyvinyl alcohol to coat polyethylene tubing, and the effect on platelet production and thrombogenicity was studied by W. E. Ip et al., in *Journal of Biomedical Materials Research,* Vol. 25, pages 875-887 (1991); C. H. Cholakis et al., in *Journal of Biomedical Materials Research,* Vol. 23, pages 417-441 (1989); and C. H. Gemmell et al., in *Journal of Biomedical Materials Research,* 37(2), pages 176-181 (1997). These references disclose that polyvinyl alcohol is not thromboadherent, but it is thrombogenic. The use of immobilized heparin does not appear to have any additional effect on thrombogenicity.

U.S. Patent No. 4,871,357 and U.S. Patent No. 5,417,969 describe anti-thrombogenic surface coatings for plastic medical devices. The coatings are prepared by complexing heparin with cationic organic molecules, such as alkylbenzyl dimethyl ammonium salts. The coating can be fixed to the plastic substrate using ionizing radiation to form covalent bonds between the substrate and the coating. Other heparin coatings on medical devices are disclosed in U.S. Patent No. 5,679,659 and U.S. Patent No. 5,672,638,

which describe the reaction of heparin with periodate, and contacting this reaction mixture with immobilized free amine groups on a blood-contacting substrate.

Implanted devices, and surrounding tissue, can also suffer from mineralization, and more particularly calcification, due to the reaction of the body to the implant. Calcification of heart valve leaflets is one of the main reasons for prostheses failure in humans. Calcification of heart valves can lead to incompetent valve performance that can result in congestive heart failure, thrombosis and/or stroke. Carpentier et al., *The Society of Thoracic Surgeons*, pages S332 to S338 (1995), describe the use of iron pretreatment of tissues preserved in glutaraldehyde to inhibit calcification. The calcification of porcine bioprosthetic valve tissue was found to correlate directly with the iron content within the tissue. Vyavahare et al., *American Journal of Pathology*, 155(3), pages 973-982 (1999), describe the pretreatment of elastin with $AlCl_3$ to provide resistance against calcification as a result of the binding of the aluminum ions to the elastin.

It will be appreciated that a need exists for modifying the surface of a medical device or instrument for improved, longer acting utility when contact with blood or plasma, as well as improved resistance against mineralization, following a medical procedure in which the device is installed in a subject.

## SUMMARY OF THE INVENTION

In general terms, the present invention features a substrate having its surface modified with hyaluronic acid. The substrate can be a polymeric material, preferably a polyalkylene terephthalate, such as polyethylene terephthalate, or a metal such as stainless steel. The hyaluronic acid is chemically bound to the surface of the substrate to provide a hydrophilic, anti-platelet, anti-fouling surface. This is of particular advantage when the substrate is part of a medical device or instrument which contacts a biological fluid, such as blood or plasma, and the device or instrument is used in a surgical procedure performed on a patient. The surface of the substrate can be further modified to incorporate $Fe^{+3}$ or $Al^{+3}$ cations to prevent calcification of the device or instrument, and the surrounding tissue.

In one aspect, the modified substrate can be prepared by contacting the unmodified substrate with an activated hyaluronic acid in an aqueous solution under conditions sufficient to form a strong chemical bond between the substrate and the hyaluronic acid. The conditions can vary, but will generally include temperatures in the range of from about 4°C to

about 35°C, preferably from about 4°C to about 25°C, and reaction time periods of from about 2 hours to about 24 hours.

In a further aspect, the activated hyaluronic acid is formed by reacting the hyaluronic acid with a carbodiimide in an aqueous medium under suitable reaction conditions. The preferred pH for carrying out the reaction is from about 3.5 to 8.0, and more preferably 4.0 to 5.1. The preferred concentration for the hyaluronic acid is from about 0.05% w/w to about 2% w/w, more preferably from about 0.1%w/w to about 1%w/w. The molar ratio of carbodiimide to hyaluronic acid is preferably in the range of from about 0.5:1 to about 2:1, with a ratio of 1.5:1 being particularly preferred. The molecular weight of the hyaluronic acid is typically in the range of 25,000 daltons to about 2 million daltons. The preferred carbodiimide is either 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide or 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide methiodide.

In another aspect, the hyaluronic acid is modified to incorporate an amount of $Fe^{+3}$ or $Al^{+3}$ cations sufficient to inhibit the mineralization, and particularly calcification, of the substrate when in contact with a source of calcium ions. The hyaluronic acid can be modified by contacting a solution of the hyaluronic acid with a soluble ferric or aluminum salt. Preferred salts include ferric chloride, aluminum chloride, ferric citrate, aluminum citrate, and the like. Alternatively, the hyaluronic acid can be contacted with the ferric or aluminum salt after immobilization onto the surface of a substrate.

In yet another aspect, this invention relates to medical devices and instruments which have been modified as provided herein to produce surfaces which have improved hydrophilic, anti-fouling and anti-platelet adhesion characteristics, as well as improved resistance to calcification. These medical devices and instruments can be prosthetic devices, typically synthetic or bioprosthetic devices, including stents, grafts, sutures, catheters, tubings, and guidewires. Optionally, the hyaluronic acid surface layer can include a pharmaceutically active substance dispersed throughout the hyaluronic acid, making the device or instrument useful for drug delivery applications. Suitable pharmaceutically active substances include proteins, such as growth factors and enzymes, drugs, antibodies, biopolymers, and biologically compatible synthetic polymers.

It has been discovered that a medical device or instrument having a modified surface as provided herein has several advantages in comparison to an unmodified device or instrument, particularly when used in an environment in which contact with a biological fluid, such as blood or plasma, is necessitated. Such modified devices and instruments have

enhanced hydrophilicity, as well as improved anti-platelet and anti-fouling characteristics, and improved resistance to calcification. Since the hyaluronic acid is chemically bound to the surface of the device or instrument, it forms a durable and stable coating, as contrasted to a simple physical coating. Further, HA is a biopolymer, and is less likely to exhibit an unfavorable biological response than a synthetic polymer, such as polyvinyl alcohol. Suitable devices and instruments include prosthetic devices, such as coronary valves and vascular grafts, bioprosthetic devices, stents, catheters, tubes, sutures, guidewires, films, and fabrics.

The devices and instruments of this invention, when used in a surgical procedure performed on a human patient, can result in a reduction in thrombosis, as well as a reduction in complications resulting from thrombosis, a reduction in tissue damage, increased resistance to bacterial adhesion, a resistance to cell binding, and a reduction in adhesion formation. Moreover, the reduction in platelet adhesion and aggregation can be accomplished without interfering with other hemostatic events, such as thromboxane production, which is a potent regulator of normal platelet function, and fibrinolytic activity, which induces lysis of clots in the general circulation system.

The devices and instruments of the invention have been found to decrease the risk of pathological thrombus formation associated with a diseased state or a medical procedure including cardiovascular surgery, cardiopulmonary bypass, catheterization (e.g., cardiac catheterization, or angioplasty) with a substantially reduced risk of affecting overall hemostasis. Resistance to calcification allows the devices and instruments to function effectively for a prolonged period of time without adverse biological consequences.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any method and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned hereunder, including published patent applications, and issued or granted patents, are incorporated herein by reference in their entirety. Unless mentioned otherwise, the techniques employed or contemplated herein are standard methodologies well known to one of ordinary skill in the art. The materials, methods and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments, and from the claims.

## BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 illustrates the Fourier transformed infrared spectroscope (FT-IR) of an unmodified polyethylene terephthalate surface.

FIG. 2 illustrates the Fourier transformed infrared spectroscope (FT-IR) of a polyethylene terephthalate surface modified by reaction with hyaluronic acid.

FIG. 3 illustrates the HPLC trace of a glucosamine standard (RT of 15.5 and 15.8 minutes) and aminopropanol (RT of 18.7 minutes).

FIG. 4 illustrates the HPLC trace of an acid hydrolysate from a polyethylene terephthalate surface modified by reaction with hyaluronic acid showing a glucosamine peak.

FIG. 5 illustrates the glucosamine analysis using an HPLC trace of a polyethylene teraphthalate surface modified by reaction with hyaluronic acid at different time points.

FIG. 6 illustrates the electron spectroscopy for chemical analysis (ESCA) of a polyethylene teraphthalate surface modified by reaction with hyaluronic acid at different time points.

FIG. 7 illustrates the Fourier transformed infrared spectroscope (MIR-FTIR) of a polyethylene terephthalate surface modified by reaction with hyaluronic acid at Day 0.

FIG. 8 illustrates the Fourier transformed infrared spectroscope (MIR-FTIR) of a polyethylene terephthalate surface modified by reaction with hyaluronic acid at Day 62.

FIG. 9 is a bar graph depicting cell attachment for unmodified polyethylene terephthalate, aminated polyethylene terephthalate, and a polyethylene terephthalate surface-modified by reaction with hyaluronic acid.

FIG. 10 is a scanning electron microscope (SEM) image of an unmodified polyethylene terephthalate surface after exposure to whole blood.

FIG. 11 is a scanning electron microscope (SEM) image of an aminated polyethylene terephthalate surface after exposure to whole blood.

FIG. 12 is a scanning electron microscope (SEM) image of a polyethylene terephthalate surface-modified by reaction with hyaluronic acid after exposure to whole blood.

FIG. 13 is a graphic representation of platelet deposition on unmodified stainless steel tubes an on hyaluronic acid-modified stainless steel tubes.

## DETAILED DESCRIPTION

The medical devices and instruments of the present invention can be prepared generally as described herein. The surface portion of the device or instrument, or a part thereof, is modified to present reactive sites which are capable of reacting with hyaluronic acid which has been suitably activated. The preferred reactive sites are primary amino groups which are formed on the substrate by amination techniques which are known in the art. Such techniques can be used to aminate plastic films and fabrics, as well as stainless steel tubes and wires. Suitable aminated substrate materials of this general description are also available commercially from various sources, such as Advanced Surface Technologies of Billerica, MA. Substrate materials include metallic materials typically used in surgical procedures, such as stainless steel, and the like, as well as polymeric materials, such as polyalkylene therephthalates, polypropylene, polyurethane, and the like.

By "polyalkylene terephthalates" is generally meant polyethylene terephthalate, polypropylene terephthalate, polybutylene terephthalate, and the like. "PET" refers to polyethylene terephthalate.

As used herein, and unless otherwise indicated, the terms "HA" and "hyaluronic acid" denote hyaluronic acid and any of its hyaluronate salts, including, for example, sodium hyaluronate (the sodium salt), potassium hyaluronate, magnesium hyaluronate, and calcium hyaluronate.

A "modified substrate" is a substrate which has its surface modified to be capable of chemically bonding with hyaluronic acid. An example of a modified substrate is a substrate which has been "aminated" and subsequently reacted with an "activated" hyaluronic acid molecule.

A substrate is "aminated" when it is chemically modified to present a reactive amino group on its surface. The amino group can be a primary or secondary amine, although primary amines are preferred. As an example of an amination technique within the scope of this invention, a substrate can be treated with radio frequency plasma deposition followed by adsorption with polyethyleneimine.

Hyaluronic acid is "activated" by reaction with an activating agent, such as a carbodiimide, or by reaction with a suitable crosslinking agent.

An "activating agent" is a substance that, in an aqueous mixture including hyaluronic acid, renders the carboxyl groups on the hyaluronic acid vulnerable to nucleophilic attack.

The terms "chemically bound" or "chemical bond" mean a covalent bond which is formed between the modified substrate and the hyaluronic acid. "HA-PET" refers to

hyaluronic acid chemically bound to polyethylene terephthalate; "HA-Dacron™" refers to hyaluronic acid chemically bound to Dacron™; "HA-S.S." refers to hyaluronic acid chemically bound to stainless steel.

An "acyl derivative," as may be used herein, is a compound produced by the displacement of the hydroxyl group bound to the acyl carbon atom of a carboxylic acid moiety by either the reaction of the hydroxyl group with a nucleophilic group of another compound, or by the rearrangement of the O-acylisourea formed by reaction of the hydroxyl group with a carbodiimide. Examples of acyl derivatives include acylureas, acylisoureas, amides, thioesters, and phenolates.

The term "mineralization" means the formation of mineral deposits on the surfaces of medical devices or instruments, or in tissue surfaces adjacent to the device after implantation in a subject. As used in a biomedical context, the term "mineralization" generally denotes "calcification", or the formation of calcium salt deposits on a surface.

The term "platelet aggregation" as used herein means the amassing together of individual platelets through specific interactions between platelets.

The term "platelet adhesion" as used herein means the amassing of platelets onto a surface (e.g., a vascular wall, prosthetic device) through interactions of the platelets with the surface.

The substrate can be a polymeric material or a metal, preferably a polyalkylene terephthalate, such as polyethylene terephthalate, or a metal such as stainless steel. The hyaluronic acid is chemically bound to the surface of the modified substrate to provide a hydrophilic, anti-platelet, anti-fouling surface. This is of particular advantage when the substrate is part of a medical device or instrument which contacts a biological fluid, such as blood or plasma. The finished substrate can be prepared by contacting the modified substrate with an activated hyaluronic acid in an aqueous solution under conditions sufficient to form a strong chemical bond between the modified substrate and the hyaluronic acid. The conditions can vary, but will generally include temperatures in the range of from about 4°C to about 35°C, preferably from about 4°C to about 25°C, and reaction time periods of from about 2 hours to about 24 hours.

The activated hyaluronic acid can be prepared by treating a hyaluronic acid with a suitable carbodiimide in the presence or absence of a nucleophile. The resulting product may be water soluble or water insoluble, depending on the reaction conditions and the relative proportions of ingredients in the reaction mixture.

The reaction of the carbodiimide with the carboxyl group of the hyaluronic acid proceeds through the addition of the free carboxylate to one of the double bonds of the diimide to give the O-acylisourea derivatives of hyaluronic acid and the carbodiimide. In the presence of a nucleophile, such as a primary amine, the amide derivative of the hyaluronic acid forms, as well as the unimolecular O→N rearrangement of the O-acylisourea derivative, to give the more stable N-acylurea derivative of the hyaluronic acid and the carbodiimide. In the absence of a nucleophile, the intramolecular rearrangement from the O-acylisourea derivative to the N-acylurea derivative is the predominant reaction.

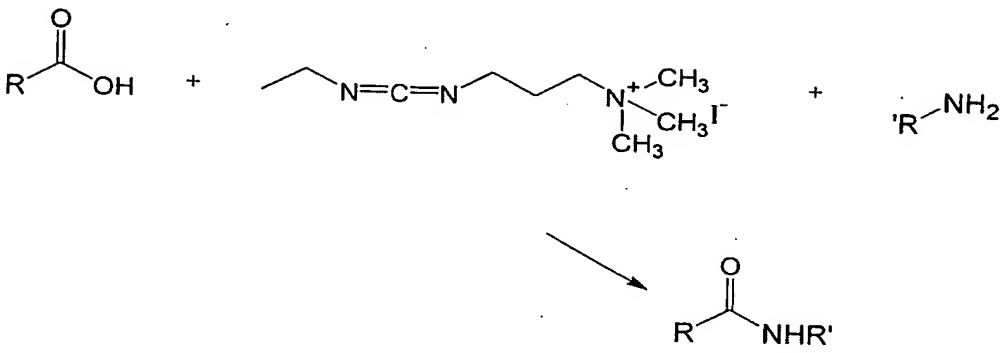
The hyaluronic acid, or a salt of hyaluronic acid, such as sodium hyaluronate, is dissolved in water to make an aqueous mixture. HA from any of a variety of sources can be used. As is well known to those skilled in the art, HA can be extracted from animal tissues or harvested as a product of bacterial fermentation. Hyaluronic acid can be produced in commercial quantities by bioprocess technology, as described for example in PCT Publication No. WO 86/04355. Preferably the concentration of HA in this first aqueous mixture is in the range of between 0.05% to 2.0% weight/weight ("w/w"), and more preferably from 0.1% to 1%. Subsequent reactions are slower and less effective at significantly lower concentrations, while significantly higher concentrations are difficult to handle due to their high viscosity. The aqueous HA mixture should initially be acidic, preferably with a pH between pH 3.5 and pH 7.01, more preferably between a pH 4.0 and 5.1. As the reaction proceeds, it becomes more basic over time. At lower pH values, the preferred activating agent, EDC, is unstable, and at higher values the reaction rate is diminished. Preferably hydrochloric acid is added to adjust the pH, although other known acids can be used.

After the addition of the carbodiimide to the aqueous solution of HA, the pH of the reaction mixture is adjusted. Preferred carbodiimides include EDC (in some references this substance is termed 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide or "DEC") or ETC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide methiodide).

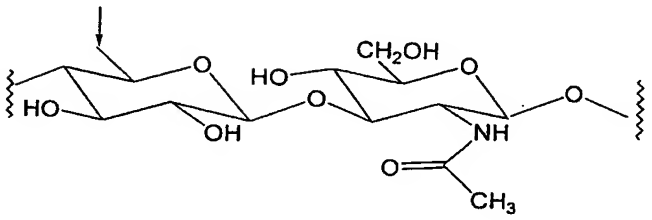
We have found that the best results are obtained when the ratio of carbodiimide to HA ranges from about 0.5:1 to 2:1, and preferably is about 1.5:1.

Suitable procedures for preparing coatings of the general type as those employed herein, and for using HA to inhibit platelet aggregation, can be found in U.S. Patents Nos. 5,527,893 and 5,585,361, respectively, the disclosures of which are incorporated by reference herein.

The activated hyaluronic acid can then be immobilized onto a solid substrate, preferably in a one step procedure, by contacting the substrate with the activated hyaluronic acid in an aqueous solution. The substrate has been modified, such as by amination, to accommodate the reactive carboxylic acid sites on the hyaluronic acid. The following illustrates the reaction of the aminated substrate, ETC and HA:



wherein R is



and R' represents the substrate.

The chemical bonding of HA onto the surface of the substrate can be illustrated as follows:

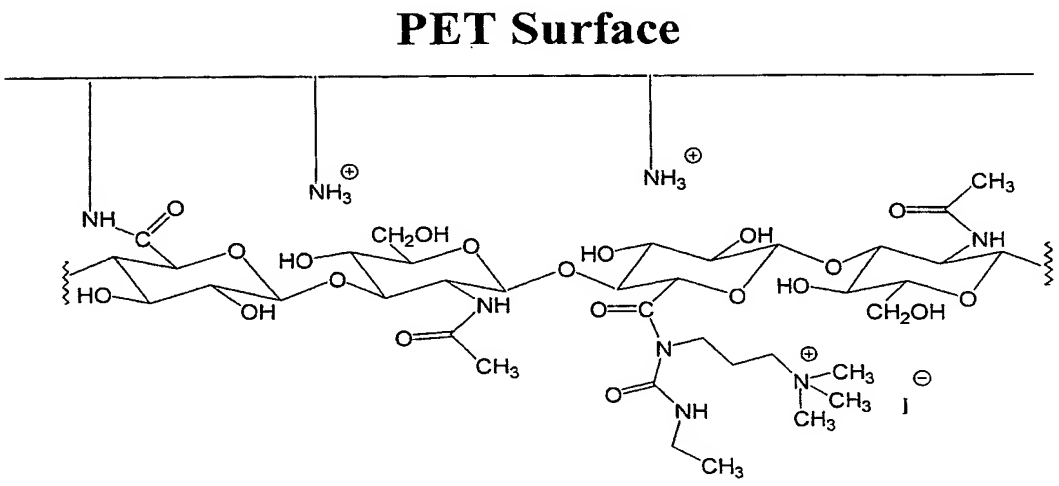



Figure 1 shows the FT/IR spectrum of the unmodified PET substrate. The surface-bound HA has attached to some of the carboxyl groups an N-acylurea group as shown by the FT/IR spectrum in Figure 2. The peak denoted by the arrow in Figure 2 has been assigned to the asymmetric stretch of the N-acylurea carbonyl as referenced in the literature. DeLos et al., *J. Am. Chem. Soc.*, Vol. 88, page 1013 (1967).

The immobilized hyaluronic acid can also include an amount of $Fe^{+3}$ or $Al^{+3}$ cations sufficient to impair or retard the mineralization of the implanted device and surrounding tissues. The amount of $Fe^{+3}$ or $Al^{+3}$ cations contained in the hyaluronic acid is generally in the range of from about 0.1% to about 15.0% by weight. The $Fe^{+3}$ or $Al^{+3}$ cations can be incorporated in the hyaluronic acid by the addition of soluble salts of these metals to a solution of the hyaluronic acid prior to or during the addition of the activating agent to the solution. Alternatively, the trivalent metal cations can be incorporated into the hyaluronic acid after immobilization onto the surface of the substrate by contacting the substrate with a solution of the trivalent metal salt. Trivalent metal salts which are particularly useful and well suited for this purpose include ferric chloride, aluminum chloride, ferric citrate and aluminum citrate. Combinations of trivalent iron and aluminum salts can also be used, and are effective.

The substrate of this invention is preferably part of a medical device or instrument. Typical of such devices are stents, grafts, prosthetic devices, bioprosthetic devices, vascular

grafts, tubes, natural or synthetic heart valves, films, fabrics, catheters, sutures, blood dialysis membranes, guidewires, and the like. These devices are provided with a coating of hyaluronic acid which is chemically bound to the surface of the device in accordance with the methods described herein. Upon exposure to blood, platelets will be less likely to adhere to the surface compared to non-HA coated surfaces.

The efficacy of any device may be tested prior to use by standard cell adhesion assays well known to those skilled in the art. For example, a small sample containing a platelet suspension is incubated with a device coated with HA at physiological temperatures, and then the percentage of platelets bound to the surface of the device is calculated.

From the above description, one skilled in the art can easily ascertain the essential characteristics of the present invention, and without departing from the spirit and scope thereof, can make various changes and modifications to the invention to adapt it to various usages and conditions.

In addition, the invention also includes the use of HA to deliver therapeutic drugs directly to desired sites within the body. For example, drugs can be incorporated into the HA by admixing or by immobilizing the drug by chemical attachment to the HA molecule, or by ionic interaction between the drug and the HA molecule (e.g., see Sparer et al., 1983, Chapter 6, pp. 107-119, *In Controlled Release Delivery Systems*, Roseman et al. (ed), Marcel Dekker, Inc.: New York). The HA-drug complex, or HA derivative complex, can then deliver the drug in a site-specific manner, for example to a damaged vessel wall.

As one skilled in the art will appreciate, the invention can be practiced using protocols that are within the method of the invention yet are different in particulars from those described herein.

The following examples of this aspect of the invention are given by way of illustration and are not intended to limit the invention except as set forth in the claims.

EXAMPLE 1

This example describes the preparation of HA-modified PET, HA-modified Dacron™, HA-modified stainless steel tubes.

Aminated substrates, for example, aminated PET films, aminated Dacron fabric and stainless steel (316W) tubes, are prepared by Advanced Surface Technology (Billerica, MA). In a typical experiment, a 0.5% w/w hyaluronic acid solution was prepared by dissolving 500 mg of hyaluronic acid (sodium salt) in 100 gm of distilled water. To the HA solution was

added, dropwise with stirring, an aqueous solution of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide methiodide (EDCI, 750 mg in 150 ml water). Sufficient 0.5N hydrochloric acid was added to adjust the pH to about 4.5, in this example about one ml. The aminated substrate was then immersed in the hyaluronic acid coating solution, and the reaction was allowed to continue for 16 hrs at 4°C.

At the end of this period, the coated substrate was removed from the coating chamber and transferred to a washing chamber filled with distilled water. The coated substrate was washed for at least 4 hours with 2 to 3 changes of water. The coated substrate was then air-dried in a laminar flow hood and characterized for HA content by an HPLC procedure, and by standard spectrophotometric techniques, including electron spectroscopy for chemical analysis (ESCA), Fourier transformed spectroscopy (FT-IR), and scanning electron microscopy (SEM), as shown in Figures 1 to 4, and in the table below.

**ESCA Analysis**

| Element | PET | Aminated Substrate | HA-coated Substrate |
|---|---|---|---|
| C | 70.4% | 67.4 ± 0.42% | 63.8 ± 2.8% |
| N | 0 | 29.6 ± 1.3% | 8.5 ± 1.22% |
| O | 26.2% | 2.6 ± 0.12% | 26.4 ± 3.3% |
| Other | 3.5% | --- | 1.4 ± 1.2% |

The hydrophilicity of the surface was also measured by captive bubble contact angle measurements as shown in the table.

**Contact Angle Measurements**

| Sample | Contact Angle |
|---|---|
| Unmodified PET | 112 degrees +/- 1.9 |
| Aminated PET | 128 degrees +/- 7.7 |
| HA-PET | 152 degrees +/- 1.1 |

EXAMPLE 2

This example illustrates the stability of HA-PET in phosphate buffered saline solution ("PBS").

Samples of HA-PET (~1" x 3") were immersed in 45 ml centrifuge tubes filled with PBS buffer. The tubes were placed on a Nutator mixer and incubated in a 37°C oven. At 1, 3, 7, 14, 21 and 62 days, at least one sample was retrieved and analyzed. The analysis

included a glucosamine analysis by HPLC, ESCA and FT-IR. The results are shown in Figures 5 to 8.

EXAMPLE 3

This example describes the attachment of cells on HA-PET films.

HA-PET films, prepared as described in Example 1, were cut and placed in wells of 6-well plates (well diameter 35 mm). Prior to cell seeding, cell culture media with serum was added to each well and the entire plate was incubated at 37°C for two hours. Human smooth muscle cells were seeded into each well at a density of $1x10^6$ per well, and the plate was again incubated for two hours at 37°C. The films were removed and rinsed three times with Hanks Balanced Salt Solution to remove all non-adherent cells. Following rinsing, 1 ml of trypsin was added to each of the films and the total number of adherent cells counted by Coulter Counter.

Rat fibroblasts and mouse mesothelial cells were also tested.

For comparison, the same procedure was repeated for non-treated PET films and aminated PET films. Figure 9 shows that the HA surface bound less cells than either the aminated or unmodified surfaces.

EXAMPLE 4

This example describes the *in vitro* platelet deposition on unmodified, aminated and HA-PET films.

Samples of unmodified, aminated and HA-PET films were placed in individual wells of a 24-well plate. To each well was added 1 ml of freshly drawn, citrated whole blood. The plate was rotated gently on a rotator at 400-600 rpm for 45 minutes at 37°C.

The PET samples were removed and thoroughly washed with PBS buffer. The samples were fixed with a 1.25% aqueous solution of glutaraldehyde, dehydrated with increasing concentrations of ethanol, critical point dried, and examined under scanning electron microscope. The results are shown in Figures 10, 11 and 12.

EXAMPLE 5

This Example describes the effect of bovine hyaluronidase digestion on HA-PET films.

HA-PET film was incubated in a 1 mg/ml solution of bovine testes hyaluronidase (Hydase) in 30 mM sodium succinate buffer containing 120 mM of sodium chloride (pH 4) at

37°C for 24 hours. The sample was rinsed with distilled water and analyzed by ESCA. The results are shown in the table below.

| Element | Pre-Hydase (% atom) | Post-Hydase (% atom) |
|---|---|---|
| C | 63.4 | 59.0 |
| N | 7.0 | 8.9 |
| O | 29.6 | 30.0 |
| Other | | 0.63 (Cl), 1.5 (Si) |

The glucosamine content was also determined by HPLC analysis. The results are shown in the table below.

| Sample | [HA] |
|---|---|
| Pre-Hydase HA-PET | 100 ug/cm$^2$ |
| Post-Hydase HA-PET | 30 ug/cm$^2$ |

EXAMPLE 6

This example describes platelet deposition on an *ex-vivo* AV Shunt. Indium-labeled autologous baboon platelets were re-infused into a normal male baboon equipped with a surgically implanted, exteriorized silicone rubber shunt between the femoral artery and vein. HA-S.S. tubes (316 medical grade stainless steel, 3.5 mm i.d., at least 18 mm in length) were inserted into 3.2 mm i.d. Silastic tubing and interposed into the shunt system. Radio labeled platelet deposition was monitored by gamma scintillation camera, and the total number of deposited platelets was calculated by dividing the deposited platelet activity (counts/min) by the whole blood activity (counts/min/ml), and multiplying by the circulating platelet count (platelets/ml). The platelet deposition was monitored for a total of 2 hours. The results are shown in Figure 13.

What is claimed is:

## CLAIMS

1. A method of improving the characteristics of a medical device substrate comprising the steps of:

modifying the surface of the substrate to present reactive sites,

preparing an activated hyaluronic acid, and

contacting the modified substrate with the activated hyaluronic acid under conditions sufficient to form a chemical bond between the activated hyaluronic acid and the reactive sites on the substrate surface.

2. The method of claim 1 wherein the substrate is a polyalkylene terephthalate.

3. The method of claim 2 wherein the substrate is polyethylene terephthalate.

4. The method of claim 3 wherein the substrate is a film.

5. The method of claim 3 wherein the substrate is a fabric.

6. The method of claim 1 wherein the substrate is stainless steel.

7. The method of claim 6 wherein the substrate is a tube.

8. The method of claim 6 wherein the substrate is a stent.

9. The method of claim 1 wherein the activated hyaluronic acid is contacted with the substrate in an aqueous solution at a temperature in the range of from about 4°C to about 35°C.

10. The method of claim 9 wherein the temperature is in the range of from about 4°C to about 25°C .

11. The method of claim 9 wherein the activated hyaluronic acid is contacted with the substrate for a time period of from about 2 hours to about 24 hours.

12. The method of claim 1 wherein the reactive site is a primary or secondary amino group.

13. The method of claim 12 wherein the reactive site is a primary amino group.

14. The method of claim 13 wherein the activated hyaluronic acid is prepared by reacting the hyaluronic acid with a carbodiimide in an aqueous solution under conditions sufficient to produce said activated hyaluronic acid.

15. The method of claim 14 wherein the reaction is carried out at a pH of from about 3.5 to about 7.0.

16. The method of claim 15 wherein the hyaluronic acid is present in the solution in a concentration in the range of from about 0.05% w/w to about 2% w/w.

17. The method of claim 16 wherein the hyaluronic acid is present in the solution in a concentration in the range of from about 0.1% w/w to about 1% w/w.

18. The method of claim 15 wherein the molar ratio of carbodiimide to hyaluronic acid is in the range of from about 0.5:1 to about 2:1.

19. The method of claim 18 wherein the molar ratio of carbodiimide to hyaluronic acid is about 1.5:1.

20. The method of claim 14 wherein said carbodiimide is 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, or 1-ethyl-3-(dimethylaminopropyl) carbodiimide methiodide.

21. The method of claim 14 wherein the hyaluronic acid has a molecular weight in the range of from about 25,000 daltons to about 2,000,000 daltons.

22. The method of claim 1 wherein the medical device is a surgical device.

23. The method of claim 1 wherein a drug substance is incorporated into the activated hyaluronic acid.

24. The method of claim 23 wherein the drug substance is selected from the group consisting of proteins, growth factors, antibodies, enzymes, drugs, biopolymers, and biologically compatible synthetic polymers.

25. A medical device or instrument for use in a human patient having an immobilized hyaluronic acid chemically bound to the surface thereof.

26. The device of claim 25 wherein the immobilized hyaluronic acid contains an amount of $Fe^{+3}$ or $Al^{+3}$ cations sufficient to retard mineralization of the device or surrounding tissue.

27. The device of claim 26 wherein the mineralization is calcification.

28. The device of claim 27 which is a prosthetic device.

29. The device of claim 28 wherein said prosthetic device is synthetic.

30. The device of claim 28 wherein said prosthetic device is bioprosthetic.

31. The device of claim 28 wherein said prosthetic device is a coronary valve.

32. The device of claim 25 which is a stent.

33. The device of claim 25 which is a vascular graft.

34. The device of claim 25 which is a catheter.

35. The device of claim 25 which is a tube.

36. The device of claim 25 which is a suture.

37. The device of claim 25 which is a guidewire.

38. An improved method of performing a surgical procedure on a patient in need of surgery, the improvement comprising employing the medical instrument or device of claim 25 in said procedure.

39. The method of claim 38 wherein the patient experiences a reduction in thrombosis or complications resulting from thrombosis as a result of the surgery.

40. The method of claim 38 wherein the patient experiences a reduction in tissue damage as a result of the surgery.

41. The method of claim 38 wherein the patient experiences an increased resistance to bacterial adhesion as a result of the surgery.

42. The method of claim 38 wherein the patient experiences a reduction in cell binding as a result of the surgery.

43. The method of claim 38 wherein the patient experiences a reduction in surgical adhesions as a result of the surgery.

44. The method of claim 38 wherein the patient experiences a reduction in tissue calcification as a result of the surgery.

45. The medical device or instrument of claim 25 wherein the hyaluronic acid inhibits the adherence of platelets to the surface of the device or instrument.

46. The medical device or instrument of claim 25 wherein the hyaluronic acid inhibits the attachment of cells to the surface of the device or instrument.

47. The medical device or instrument of claim 25 wherein the $Fe^{+3}$ or $Al^{+3}$ cations inhibit the calcification of the device or the surrounding tissue.

48. The medical device or instrument of claim 25 wherein a drug substance is incorporated in the hyaluronic acid.

49. The method of claim 48 wherein the drug substance is selected form the group consisting of proteins, growth factors, enzymes, drugs, biopolymers, and biologically compatible synthetic polymers.

50. A method of improving the mineralization resistance of a medical device substrate comprising the steps of:

covalently binding hyaluronic acid to the surface of the substrate, and

contacting the substrate with a source of $Fe^{+3}$ or $Al^{+3}$ cations.

51. The method of claim 50 wherein the medical device or instrument is implanted in a subject, and the surround tissue is also resistant to calcification.

52. The method of claim 50 wherein the source of $Fe^{+3}$ or $Al^{+3}$ cations is a solution of a trivalent iron or aluminum salt.

53. The method of claim 52 wherein the trivalent iron or aluminum salt is selected from the group consisting of ferric chloride, aluminum chloride, ferric citrate, and aluminum citrate.

54. A method of improving the calcification resistance of a medical device or instrument, or tissue surrounding the implantation site of the medical device or instrument, comprising the steps of:

preparing an activated hyaluronic acid containing $Fe^{+3}$ or $Al^{+3}$ cations, and

covalently binding the hyaluronic acid to the surface of the medical device or instrument.

55. A mineralization-resistant medical device or instrument having a coating thereon of an activated hyaluronic acid containing $Fe^{+3}$ or $Al^{+3}$ cations.

56. A medical device or instrument having a contact angle of at least 140° when in contact with a biological fluid.

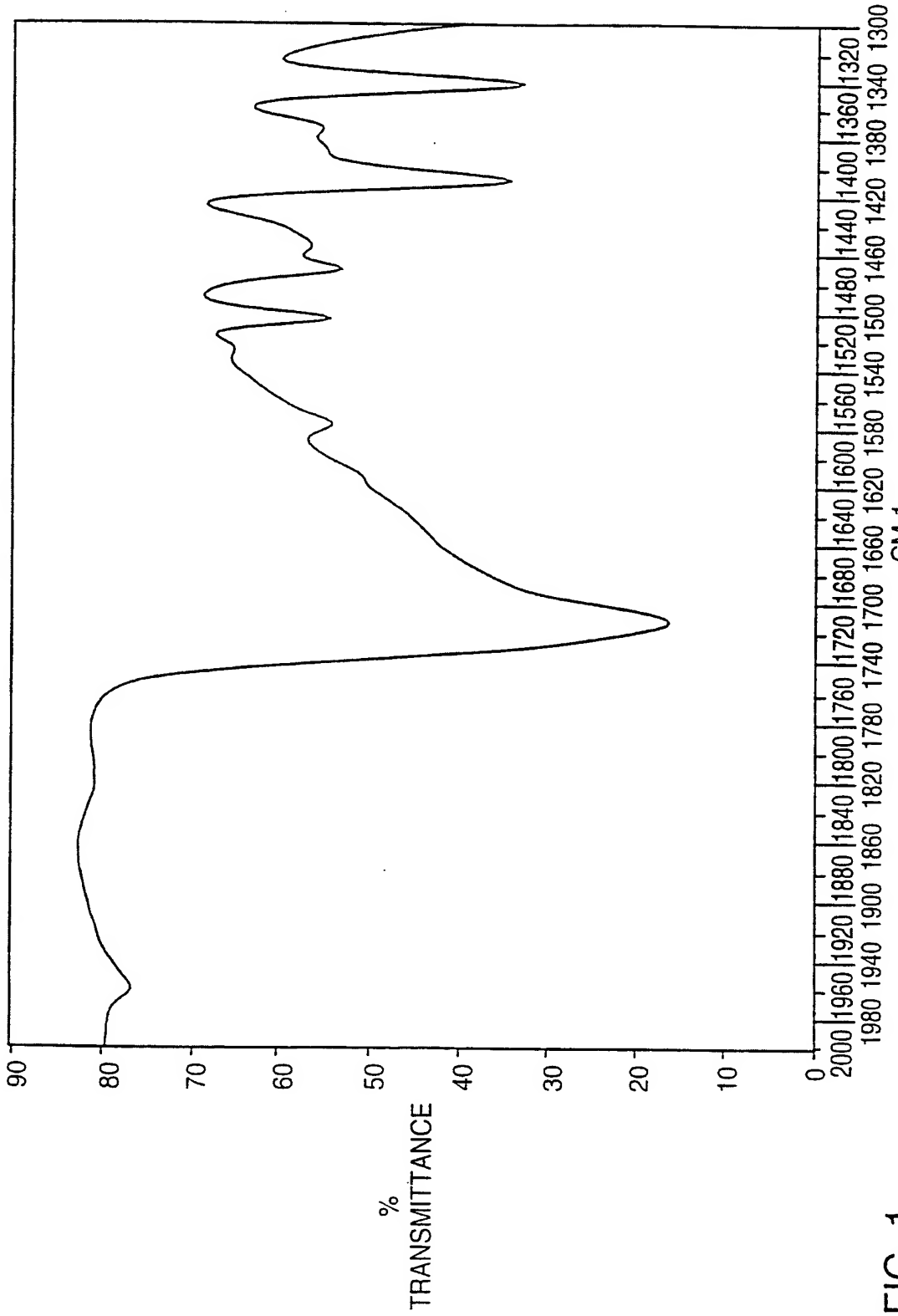

90
80
70
60
50
40
30
20
10
0
% TRANSMITTANCE
2000 1960 1920 1880 1840 1800 1760 1720 1680 1640 1600 1560 1520 1480 1440 1400 1360 1320 1300
1980 1940 1900 1860 1820 1780 1740 1700 1660 1620 1580 1540 1500 1460 1420 1380 1340
CM-1

FIG. 1

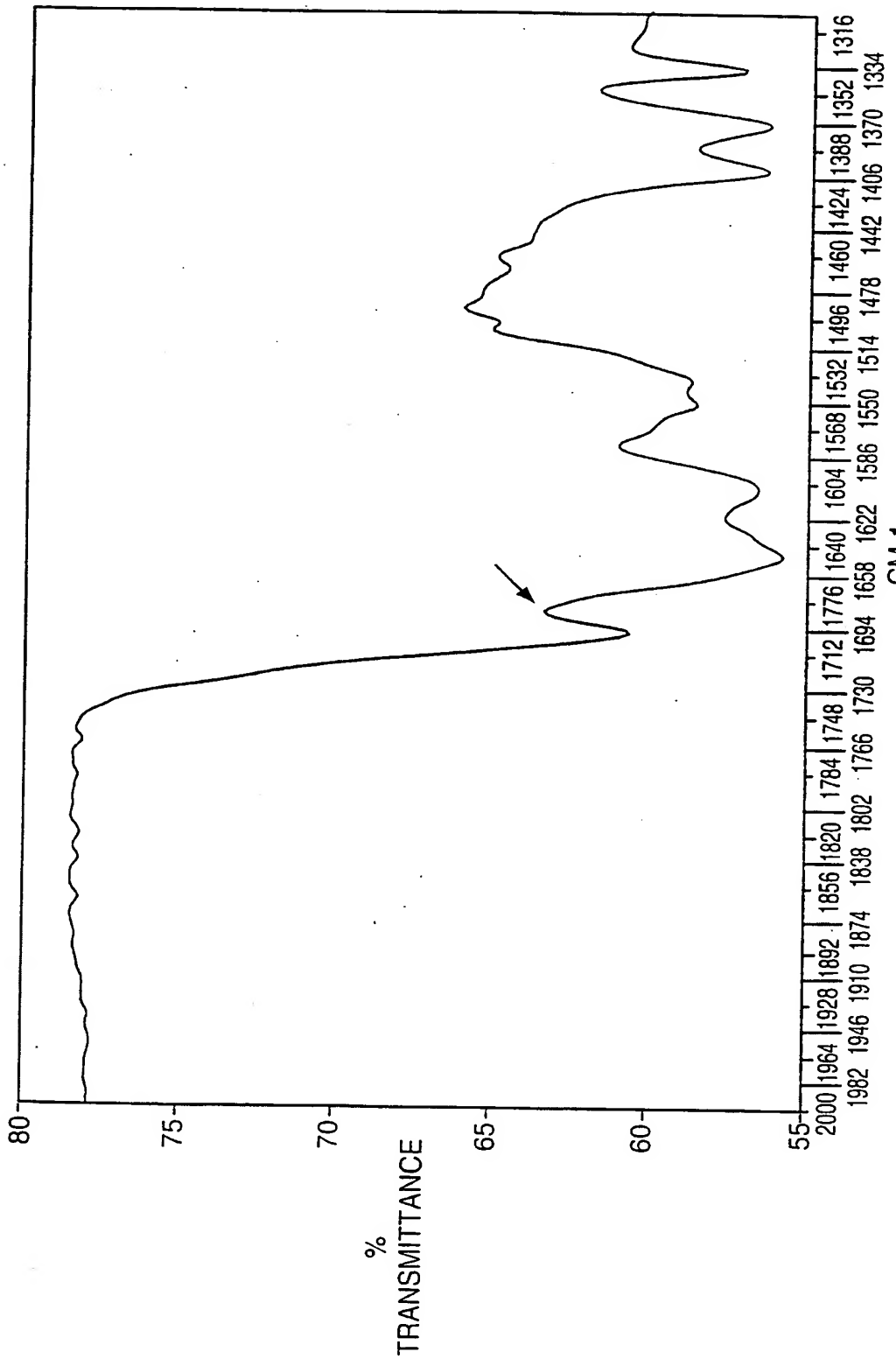

80
75
70
65
60
55
% TRANSMITTANCE
2000 1964 1928 1892 1856 1820 1784 1748 1712 1776 1640 1604 1568 1532 1496 1460 1424 1388 1352 1316
1982 1946 1910 1874 1838 1802 1766 1730 1694 1658 1622 1586 1550 1514 1478 1442 1406 1370 1334
CM-1

FIG. 2

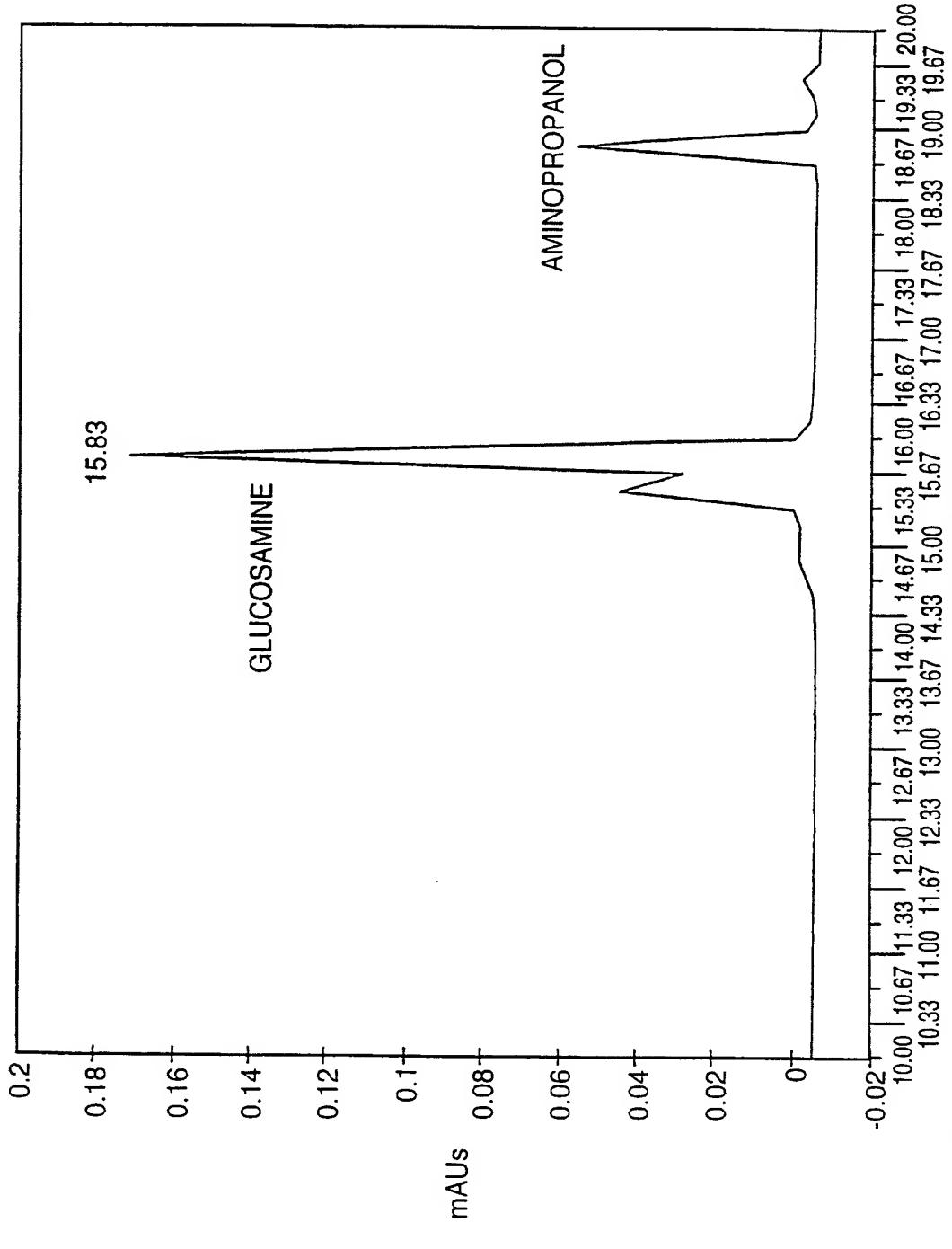

15.83
GLUCOSAMINE
AMINOPROPANOL
mAUs
0.2
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
-0.02
10.00 10.33 10.67 11.00 11.33 11.67 12.00 12.33 12.67 13.00 13.33 13.67 14.00 14.33 14.67 15.00 15.33 15.67 16.00 16.33 16.67 17.00 17.33 17.67 18.00 18.33 18.67 19.00 19.33 19.67 20.00
TIME (MIN)

FIG. 3

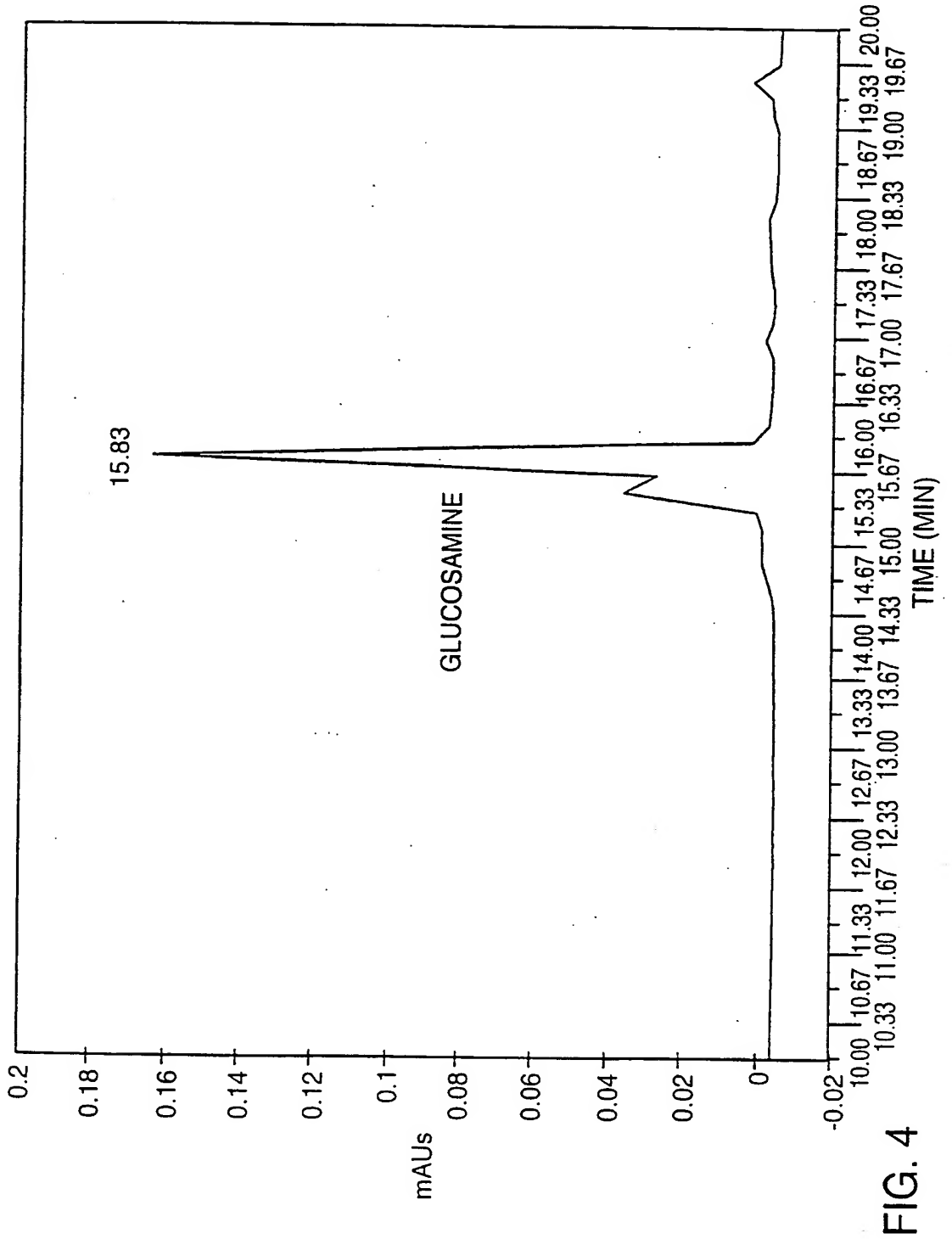

15.83
GLUCOSAMINE
mAUs
0.2
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
-0.02
10.00 10.33 10.67 11.00 11.33 11.67 12.00 12.33 12.67 13.00 13.33 13.67 14.00 14.33 14.67 15.00 15.33 15.67 16.00 16.33 16.67 17.00 17.33 17.67 18.00 18.33 18.67 19.00 19.33 19.67 20.00
TIME (MIN)

FIG. 4

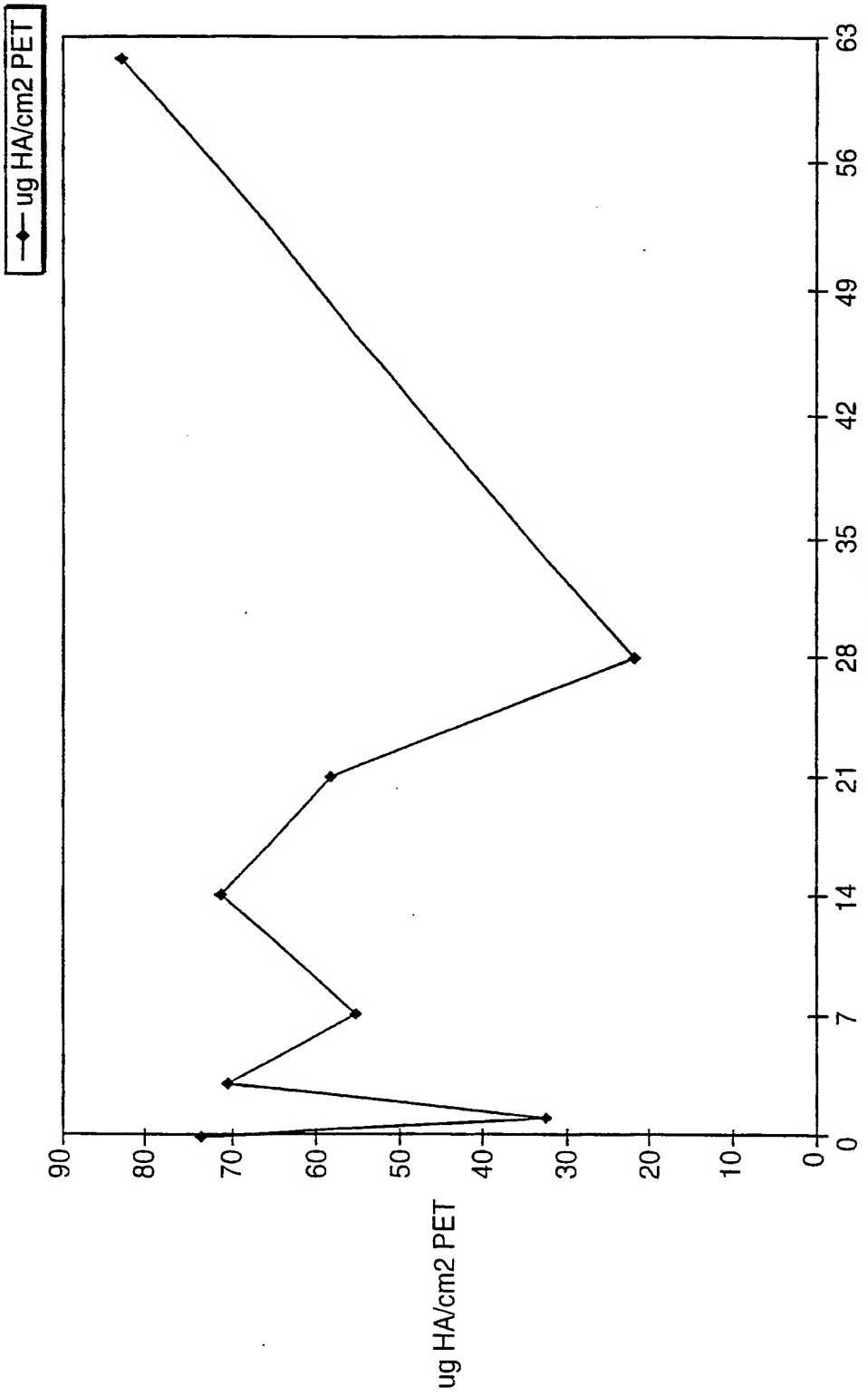

ug HA/cm2 PET
90
80
70
60
50
40
30
20
10
0
ug HA/cm2 PET
0
7
14
21
28
35
42
49
56
63
TIME (DAYS)

FIG. 5

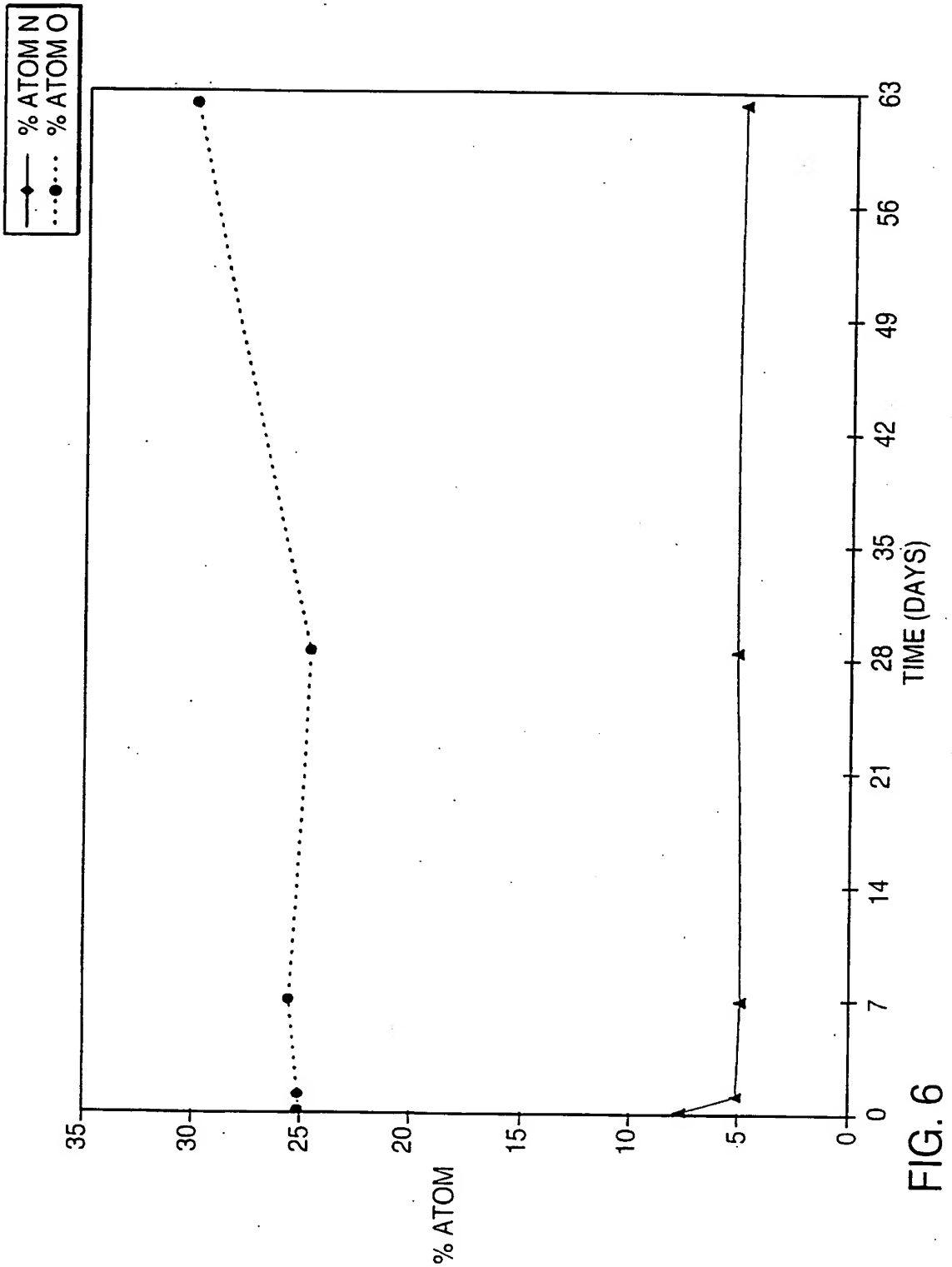

% ATOM N
% ATOM O
% ATOM
TIME (DAYS)
0
5
10
15
20
25
30
35
0
7
14
21
28
35
42
49
56
63

FIG. 6

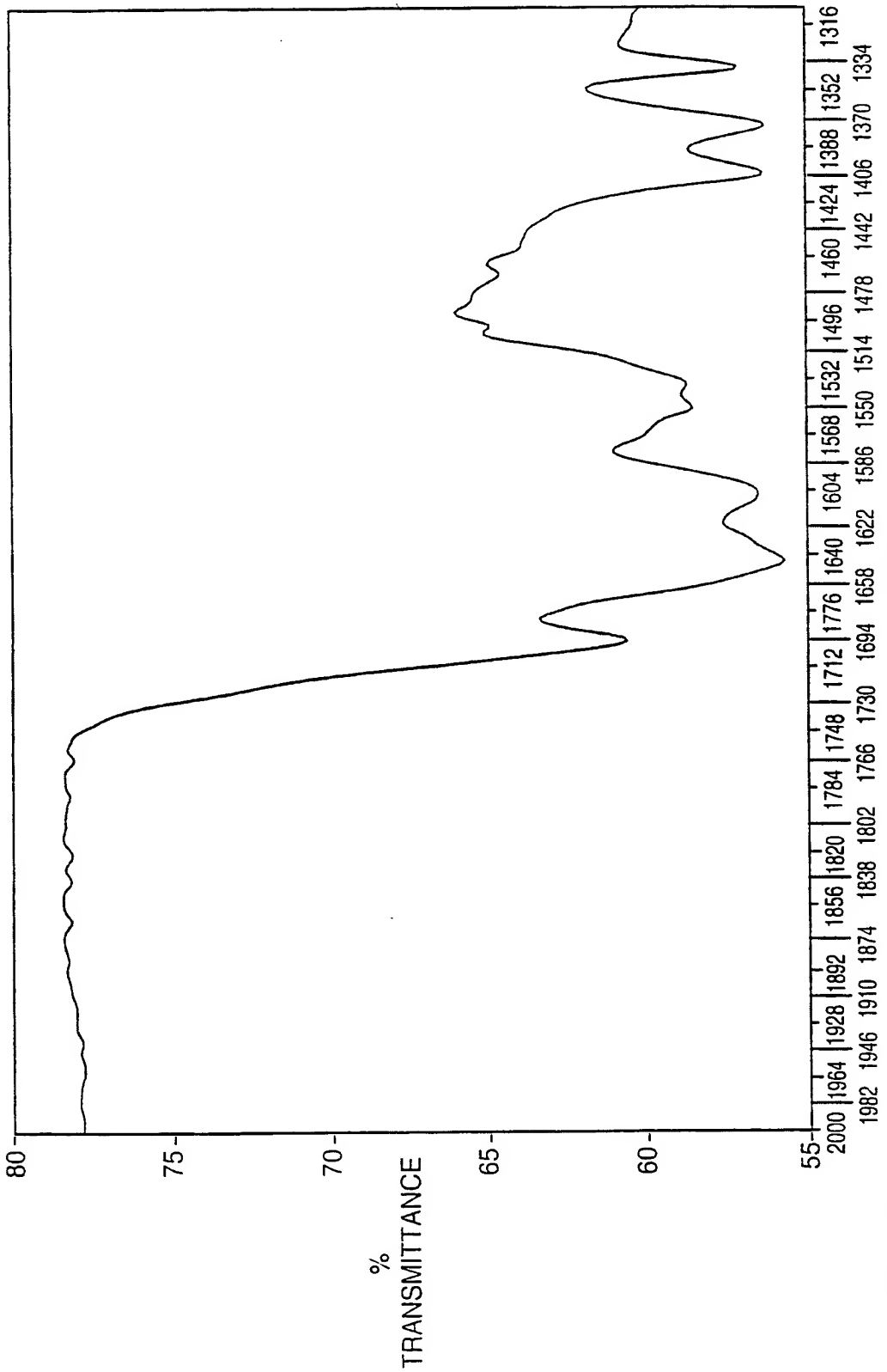

80
75
70
65
60
55
% TRANSMITTANCE
2000 1964 1928 1892 1856 1820 1784 1748 1712 1776 1640 1604 1568 1532 1496 1460 1424 1388 1352 1316
1982 1946 1910 1874 1838 1802 1766 1730 1694 1658 1622 1586 1550 1514 1478 1442 1406 1370 1334
CM-1

FIG. 7

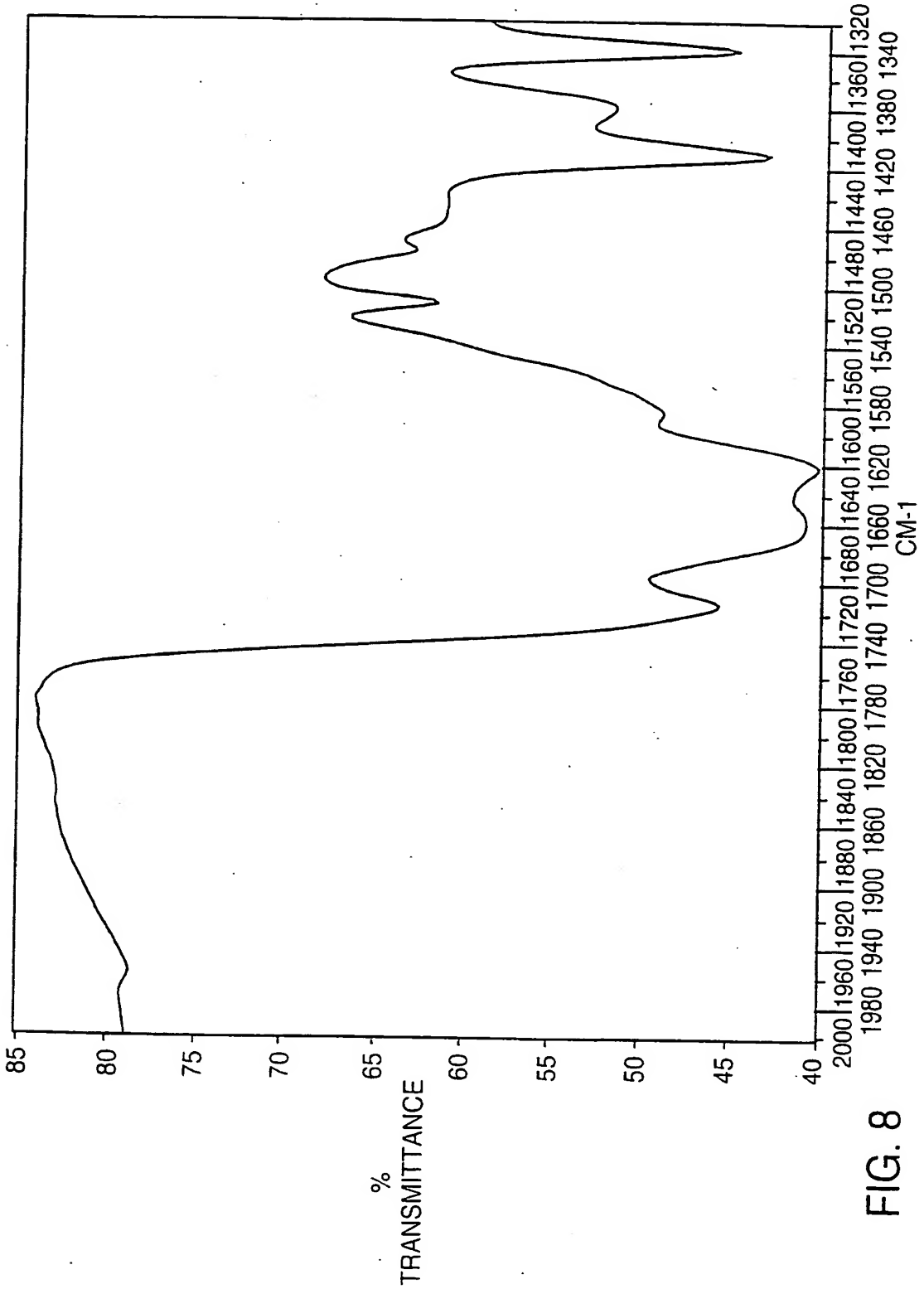

85
80
75
70
65
60
55
50
45
40
% TRANSMITTANCE
2000 1960 1920 1880 1840 1800 1760 1720 1680 1640 1600 1560 1520 1480 1440 1400 1360 1320
1980 1940 1900 1860 1820 1780 1740 1700 1660 1620 1580 1540 1500 1460 1420 1380 1340
CM-1

FIG. 8

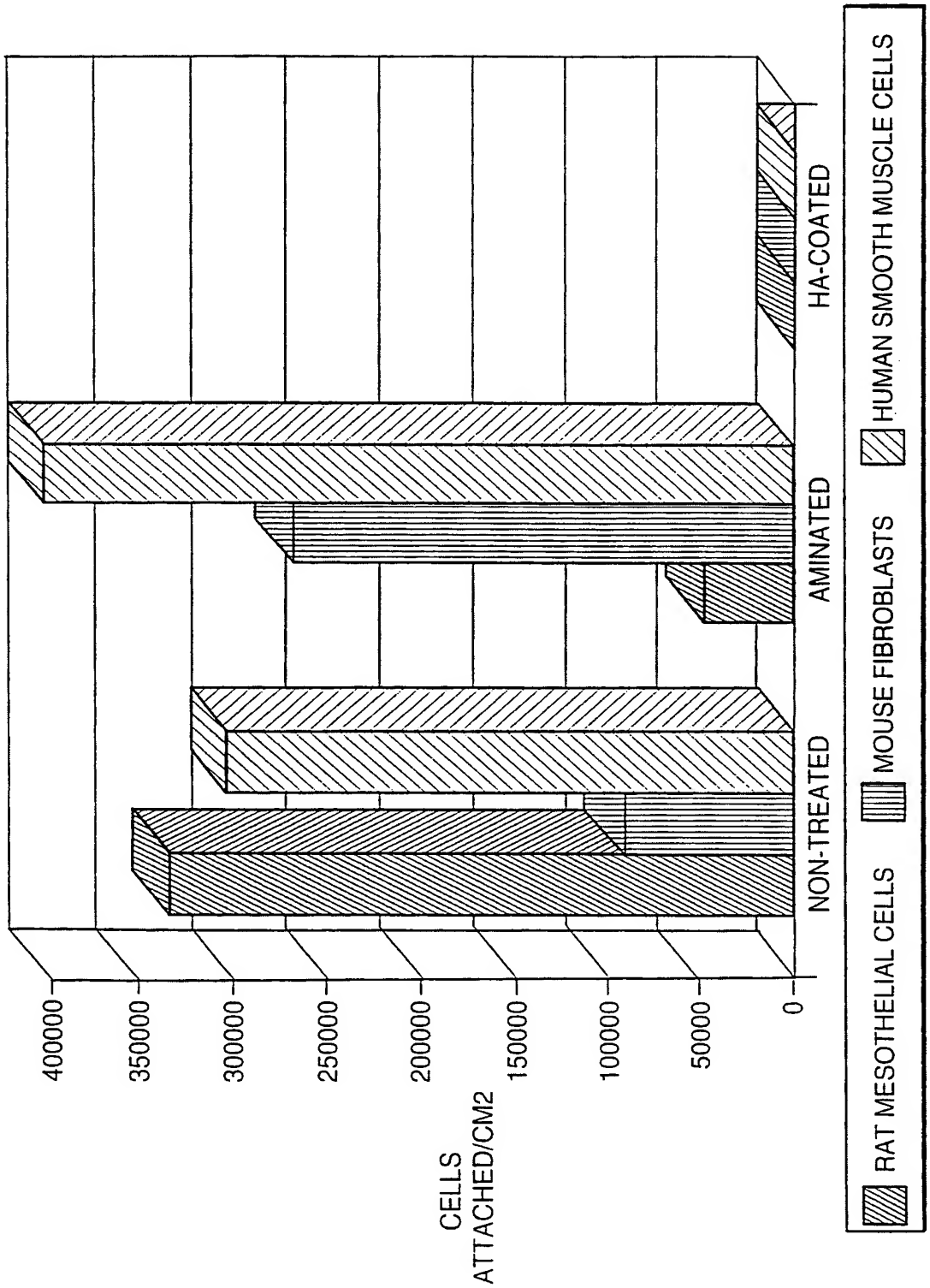

CELLS ATTACHED/CM2
400000
350000
300000
250000
200000
150000
100000
50000
0
NON-TREATED
AMINATED
HA-COATED
RAT MESOTHELIAL CELLS
MOUSE FIBROBLASTS
HUMAN SMOOTH MUSCLE CELLS

FIG. 9


FIG. 10


FIG. 11

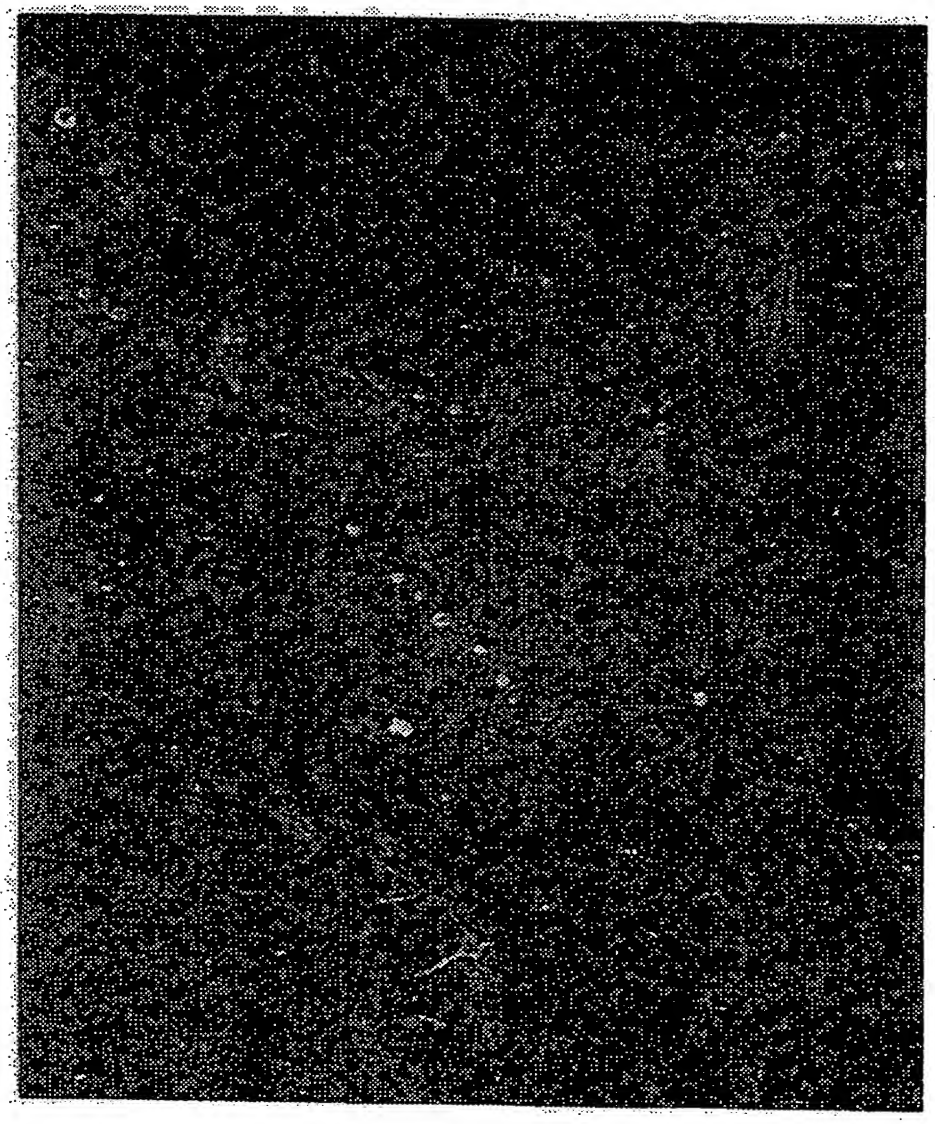



FIG. 12

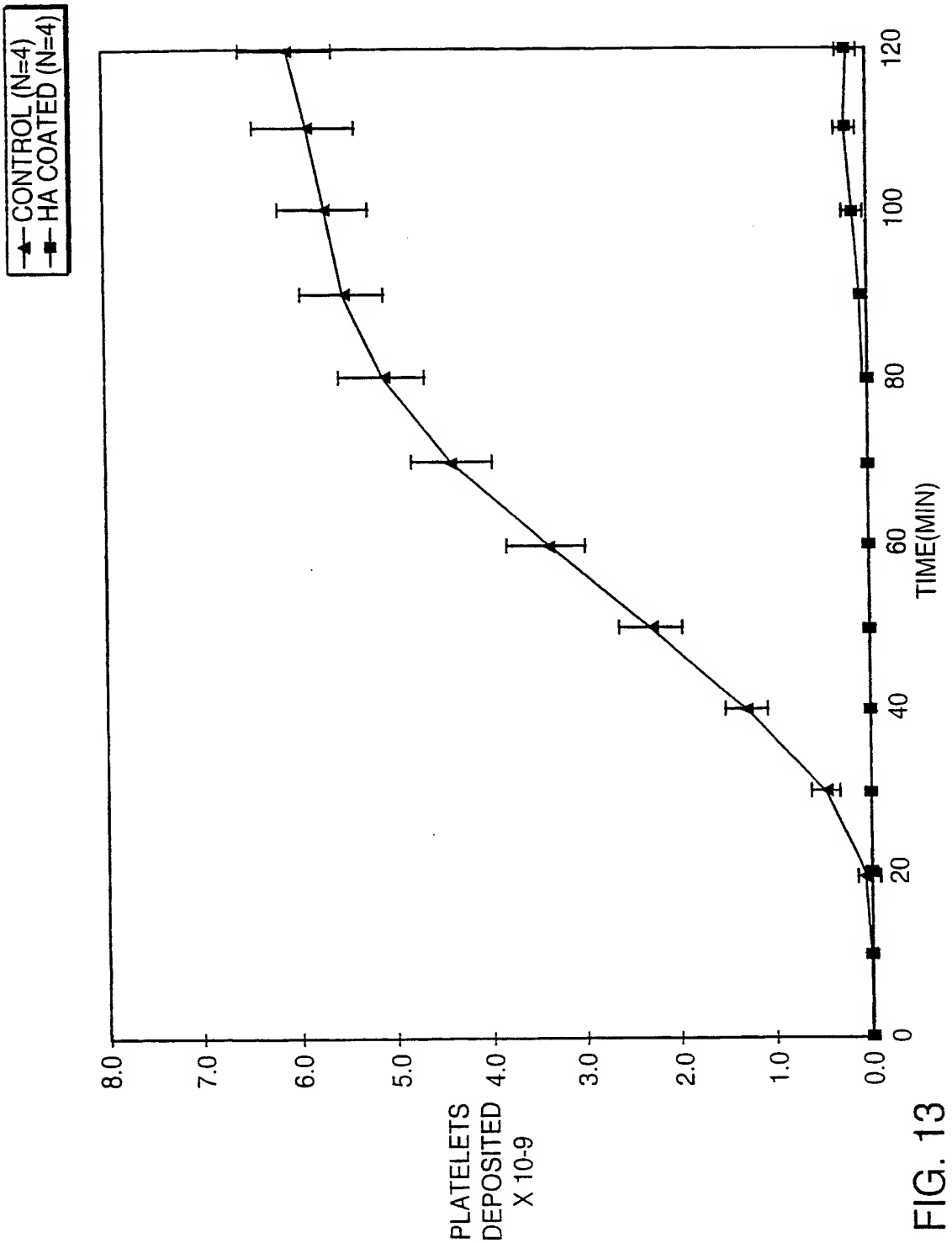

CONTROL (N=4)
HA COATED (N=4)
PLATELETS DEPOSITED X 10-9
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0
20
40
60
80
100
120
TIME(MIN)

FIG. 13

# INTERNATIONAL SEARCH REPORT

Inter ɔnal Application No
PCT/US 00/07228

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61L33/08 A61L27/34 A61L31/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, FSTA, INSPEC, COMPENDEX, MEDLINE, CHEM ABS Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | US 5 409 696 A (NARAYANAN PALLASSANA V ET AL) 25 April 1995 (1995-04-25)<br><br>column 2, line 21 - line 68<br>column 4, line 38 -column 5, line 9<br>example 1<br>--- | 1,9-15,<br>20-22,<br>25,32,<br>35-43,<br>45,46 |
| X | US 5 510 418 A (RHEE WOONZA M ET AL)<br>23 April 1996 (1996-04-23)<br><br>abstract<br>column 4, line 26 - line 58<br>column 5, line 8 - line 45<br>---<br>-/-- | 1,2,4,5,<br>9-15,<br>20-25,<br>32-43,<br>45,46,<br>48,49 |

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 23 June 2000 | 14/07/2000 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | Menidjel, R |

Form PCT/ISA/210 (second sheet) (July 1992)

# INTERNATIONAL SEARCH REPORT

Inter. nal Application No
PCT/US 00/07228

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication,where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | CHANDY T ET AL: "Structural studies on bovine bioprosthetic tissues and their in vivo calcification: prevention via drug delivery" , BIOMATERIALS,GB,ELSEVIER SCIENCE PUBLISHERS BV., BARKING, VOL. 17, NR. 6, PAGE(S) 577-585 XP004032748 ISSN: 0142-9612 page 577, right-hand column, paragraph 2 -page 578, right-hand column, paragraph 1 page 582, right-hand column, paragraph 1 --- | 25-38, 44,47, 50-56 |
| A | US 5 532 221 A (HUANG W JAMES ET AL) 2 July 1996 (1996-07-02) column 2, line 6 - line 54 column 3, line 1 - line 39 claims 1-10 --- | 25-56 |
| A | US 4 937 270 A (FOX ELLEN M ET AL) 26 June 1990 (1990-06-26) column 2, line 29 -column 3, line 60 claims 1-8 --- | 1,9-24 |
| P,X | MASON M., VERCRUYSSE K.P., KIRKER K.R., FRISCH R., MARECAK D.M., PRESTWICH G.D., PITT W.G.: "Attachment of hyaluronic acid to polypropylene, polystyrene and polytetrafluoroethylene" BIOMATERIALS, vol. 21, no. 1, January 2000 (2000-01), pages 31-36, XP002140803 page 31, right-hand column, paragraph 2 -page 33, left-hand column, paragraph 2 ----- | 1-5, 9-15, 18-22,25 |

3

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

# INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 00 /07228

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 38-44 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the composition.

---

Continuation of Box I.1

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by surgery

---

Continuation of Box I.2

The claim 56 fails to comply with the Rule 6.3(a) PCT such an extent that a meaningful search for the claim could not be carried out. The medical device is not characterized by its own technical features. The "140° angle" feature is a feature defining its use and cannot be used to formulate a meaningful search.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter nal Application No
PCT/US 00/07228

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| US 5409696 A | 25-04-1995 | US 5132108 A | 21-07-1992 |
| | | US 5591140 A | 07-01-1997 |
| | | NL 9201301 A | 16-02-1994 |
| | | US 5486357 A | 23-01-1996 |
| | | US 5244654 A | 14-09-1993 |
| US 5510418 A | 23-04-1996 | US 5324775 A | 28-06-1994 |
| | | US 5162430 A | 10-11-1992 |
| | | CA 2134745 A | 04-05-1995 |
| | | EP 0656215 A | 07-06-1995 |
| | | JP 7278203 A | 24-10-1995 |
| | | US 5470911 A | 28-11-1995 |
| | | US 5476666 A | 19-12-1995 |
| | | US 5510121 A | 23-04-1996 |
| | | AU 677789 B | 08-05-1997 |
| | | AU 4662093 A | 31-01-1994 |
| | | EP 0648239 A | 19-04-1995 |
| | | JP 8502082 T | 05-03-1996 |
| | | WO 9401483 A | 20-01-1994 |
| | | AT 168708 T | 15-08-1998 |
| | | AU 638687 B | 08-07-1993 |
| | | AU 4660989 A | 12-06-1990 |
| | | CA 2003538 A | 21-05-1990 |
| | | DE 68928754 D | 27-08-1998 |
| | | DE 68928754 T | 14-01-1999 |
| | | EP 0444157 A | 04-09-1991 |
| | | ES 2119743 T | 16-10-1998 |
| | | JP 2505312 B | 05-06-1996 |
| | | JP 4502027 T | 09-04-1992 |
| | | WO 9005755 A | 31-05-1990 |
| | | US 5306500 A | 26-04-1994 |
| | | US 5565519 A | 15-10-1996 |
| | | US 5376375 A | 27-12-1994 |
| | | US 5413791 A | 09-05-1995 |
| | | US 5475052 A | 12-12-1995 |
| | | US 5550187 A | 27-08-1996 |
| | | US 5523348 A | 04-06-1996 |
| | | US 5446091 A | 29-08-1995 |
| | | US 5543441 A | 06-08-1996 |
| | | US 5527856 A | 18-06-1996 |
| | | US 5614587 A | 25-03-1997 |
| | | US 5550188 A | 27-08-1996 |
| | | US 5643464 A | 01-07-1997 |
| | | US 5936035 A | 10-08-1999 |
| | | US 5800541 A | 01-09-1998 |
| | | US 5786421 A | 28-07-1998 |
| | | US 5744545 A | 28-04-1998 |
| | | US 5328955 A | 12-07-1994 |
| | | US 5264214 A | 23-11-1993 |
| | | US 5308889 A | 03-05-1994 |
| | | US 5292802 A | 08-03-1994 |
| | | US 5304595 A | 19-04-1994 |
| US 5532221 A | 02-07-1996 | AU 647905 B | 31-03-1994 |
| | | AU 1401592 A | 08-10-1992 |
| | | BR 9201215 A | 01-12-1992 |
| | | CA 2065111 A | 06-10-1992 |
| | | EP 0507604 A | 07-10-1992 |

# INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter nal Application No
PCT/US 00/07228

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| US 5532221 A | | GR 92100122 A | 16-03-1993 |
| | | JP 2702641 B | 21-01-1998 |
| | | JP 5124968 A | 21-05-1993 |
| US 4937270 A | 26-06-1990 | AT 138940 T | 15-06-1996 |
| | | AU 606230 B | 31-01-1991 |
| | | AU 2482588 A | 17-04-1989 |
| | | CA 1332235 A | 04-10-1994 |
| | | DE 3855351 D | 11-07-1996 |
| | | DE 3855351 T | 10-10-1996 |
| | | DK 68990 A | 17-05-1990 |
| | | EP 0397652 A | 22-11-1990 |
| | | FI 94357 B | 15-05-1995 |
| | | JP 2670996 B | 29-10-1997 |
| | | JP 9183804 A | 15-07-1997 |
| | | JP 2684208 B | 03-12-1997 |
| | | JP 3502704 T | 20-06-1991 |
| | | NO 301770 B | 08-12-1997 |
| | | NO 942763 A | 16-03-1990 |
| | | WO 8902445 A | 23-03-1989 |
| | | US 5760200 A | 02-06-1998 |
| | | US 6030958 A | 29-02-2000 |
| | | US 5527893 A | 18-06-1996 |